CLAIMS

What is claimed is:

1. A method for determining a bacterium species, comprising:

annealing a region of a nucleotide template to a specific oligonucleotide primer set comprising SEQ-FOR and SEQ-REV in a complimentary fashion, said primer set designed to provide a product having a predetermined size dictated by a complimentary primer set;

amplifying said region of said nucleotide template to produce said product;

2. The method of claim 1, wherein said SEQ-FOR is an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof.

3. The method of claim 1, wherein said SEQ-FOR is an UB-f selected from said SEQ ID NO: 90 through SEQ ID NO: 117 or a fragment or a variation thereof, and said SEQ-REV is an UB-r, a reverse primer sequence selected from said SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

4. The method of claim 1, wherein said SEQ-FOR is AFB-f selected from SEQ ID NO: 18 through said SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an UB-r selected from SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

5. A method for determining a bacterium species, comprising:

annealing a region of a nucleotide template to a specific oligonucleotide primer set comprising SEQ-FOR and SEQ-REV in a complimentary fashion, said primer set designed to provide a product having a predetermined size dictated by a complimentary primer set;

amplifying said region of said nucleotide template to produce said product; and

determining a species of a bacterium in a nucleotide sequence of said product.

6. The method of claim 5, wherein annealing said region of said nucleotide template includes a hypervariable region of said nucleotide template.

7. The method of claim 5, wherein amplifying said region of said nucleotide template includes a polymerase chain reaction having at least approximately 15 cycles.

8. The method of claim 5, wherein said SEQ-FOR is an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is

an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof.

9. The method of claim 5, wherein said SEQ-FOR is an UB-f selected from said SEQ ID NO: 90 through SEQ ID NO: 117 or a fragment or a variation thereof, and said SEQ-REV is an UB-r, a reverse primer sequence selected from said SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

10. The method of claim 5, wherein said SEQ-FOR is AFB-f selected from SEQ ID NO: 18 through said SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an UB-r selected from SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

11. The method of claim 8, wherein a length of said product is between approximately 550 base pairs and approximately 650 base pairs.

12. The method of claim 8 or 9, wherein a length of said product is between approximately 500 base pairs and approximately 700 base pairs.

13. The method of claim 10, wherein a length of said product is between approximately 1000 base pairs and 1700 base pairs.

14. The methods of claim 5, including comparing said product and said nucleotide sequence to determine said bacterium species to be a Mycobacterium, an acid-fast bacillus, or a nocardium.

15. The method of claim 5, further comprising:

(a) providing a query of said product to a BLAST™ program;

(b) substantially matching said query and a subject to provide a nucleotide identity; and

(c) determining said bacterium species using said nucleotide identity.

16. A method for determining a bacterium species, comprising:

extracting a genomic nucleotide from a specimen to provide a nucleotide template;

annealing a region in the nucleotide template to specific oligonucleotide primer set comprising SEQ-FOR and SEQ-REV in a complimentary fashion, said primer set designed to provide to a product having a particular size dictated by the complimentary primer set;

amplifying said region of said nucleotide template to produce said product; and

determining a species of a bacterium in a nucleotide sequence of said product.

17. The method of claim 16, wherein extracting said genomic nucleotide is performed using a PrepMan™ Reagent.

18. The method of claim 16, including selecting said specimen from blood, sputum, bronchial alveolar lavage, bronchial wash, tissue biopsy, body fluids, pus, urine, bone marrow aspirate, gastric aspirate, stool, tissue fluid, implanted medical device, prosthetic device, soil, sludge, water or liquids.

19. The method of claim 16, wherein said region of said nucleotide template is a hypervariable region.

20. The methods of claim 16, including comparing said product and said nucleotide sequence to determine said bacterium species to be a Mycobacterium, an acid-fast bacillus, or a nocardium.

21. The method of claim 16, further comprising:

(a) providing a query of said product to a BLAST™ program;

(b) substantially matching said query and a subject to provide a nucleotide identity; and

(c) determining said bacterium species using said nucleotide identity.

22. The method of claim 16, wherein said SEQ-FOR is an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof.

23. The method of claim 16, wherein said SEQ-FOR is an UB-f selected from said SEQ ID NO: 90 through SEQ ID NO: 117 or a fragment or a variation thereof, and said SEQ-REV is an UB-r, a reverse primer sequence selected from said SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

24. The method of claim 16, wherein said SEQ-FOR is AFB-f selected from SEQ ID NO: 18 through said SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an UB-r selected from SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

25. The method of claim 22, wherein a length of said product is between approximately 550 base pairs and approximately 650 base pairs.

26. The method of claim 22 or 23, wherein a length of said product is between approximately 500 base pairs and approximately 700 base pairs.

27. The method of claim 24, wherein a length of said product is between approximately 1000 base pairs and 1700 base pairs.

28. A method for determining a bacterium species, comprising:

culturing a bacterium from a specimen;

extracting genomic nucleotide from said bacterium to provide a nucleotide template;

annealing a desired region in the nucleotide template to a specific oligonucleotide primer set comprising SEQ-FOR and SEQ-REV in a complimentary fashion, said primer set designed to provide a product having a particular size dictated by the complimentary primer set;

amplifying said region of said nucleotide template to produce said product;

detecting said product; and

determining a species of a bacterium from a nucleotide sequence of said product.

29. The method of claim 28 including detecting said product by a gel electrophoresis and comparing said PCR product in an ethidium bromide stained-agarose gel to a DNA molecular weight ladder to determine a size of said PCR product.

30. The method of claim 28, wherein said genomic nucleotide is extracted using a PrepMan™ Reagent.

31. The method of claim 28, wherein annealing said region of said nucleotide template includes a hypervariable region of said nucleotide template.

32. The method of claim 28, wherein amplifying said region of said nucleotide template includes a polymerase chain reaction having at least approximately 15 cycles.

33. The methods of claim 28, including comparing said product and said nucleotide sequence to determine said bacterium species to be a Mycobacterium, an acid-fast bacillus, or a nocardium.

34. The method of claim 28, further comprising:

(a) providing a query of said product to a BLAST™ program;

(b) substantially matching said query and a subject to provide a nucleotide identity; and

(c) determining said bacterium species using said nucleotide identity.

35. The method of claim 28, wherein said SEQ-FOR is an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof.

36. The method of claim 28, wherein said SEQ-FOR is an UB-f selected from said SEQ ID NO: 90 through SEQ ID NO: 117 or a fragment or a variation thereof, and said SEQ-REV is an UB-r, a reverse primer sequence selected from said SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

37. The method of claim 28, wherein said SEQ-FOR is AFB-f selected from SEQ ID NO: 18 through said SEQ ID NO: 53 or a fragment or a variation thereof, and said SEQ-REV is an UB-r selected from SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof.

38. The method of claim 35, wherein a length of said product is between approximately 550 base pairs and approximately 650 base pairs.

39. The method of claim 35 or 36, wherein a length of said product is between approximately 500 base pairs and approximately 700 base pairs.

40. The method of claim 37, wherein a length of said product is between approximately 1000 base pairs and 1700 base pairs.

41. A method of determining bacterium species, comprising:

providing a specimen having a template;

providing a pair of primers selected from a group consisting of:

(a) a first forward primer having consecutive bases of an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof and a first reverse primer having consecutive bases of an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof;

(b) a second forward primer having consecutive bases of an UB-f selected from SEQ ID NO: 90 through SEQ ID NO:117 or a fragment or a variation thereof and a second reverse primer having consecutive bases of an UB-r selected from SEQ ID NO: 118 through SEQ ID NO: 145 or a fragment or a variation thereof; and

(c) a first forward primer having consecutive bases of an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof and a second reverse primer having consecutive bases of an UB-r selected from SEQ ID NO: 118 through SEQ ID NO:145 or a fragment or a variation thereof;

amplifying a region of said template using said pair of primers to produce a product from said specimen; and

comparing said product from said specimen with a nucleotide sequence from a database to determine said bacterium species.

42. The method of claim 41, wherein said region of said template is a hypervariable region by which said product may be a species-specific nucleotide sequence derived from any combination of said PCR primers.

43. The method of claim 41, wherein said AFB-f and said AFB-r are specific for a bacterium including acid-fast bacillus by which said product may be a species-specific nucleotide sequence derived from said primers.

44. The method of claim 41, wherein said UB-f and said UB-r are universal primers for amplification a bacterium by which said product may be a species-specific nucleotide sequence derived from said primers.

45. A method of determining a bacterium species, comprising:

providing a sample having a template;

providing a forward primer having consecutive bases of an AFB-f selected from SEQ ID NO: 18 through SEQ ID NO: 53 or a fragment or a variation thereof and a reverse primer having consecutive bases of an AFB-r selected from SEQ ID NO: 54 through SEQ ID NO: 89 or a fragment or a variation thereof; and

amplifying a region of said template using said pair of primers to produce a product from said specimen; and

comparing said product from said specimen with a nucleotide sequence from a database to determine said bacterium species present in the specimen.

46. A method of determining a bacterium species, comprising:

providing a sample having a template;

providing a forward primer having consecutive bases of an UB-f selected from SEQ ID NO: 90 through SEQ ID NO:117 or a fragment or a variation thereof and a reverse primer having consecutive bases of an UB-r selected from SEQ ID NO: 118 through SEQ ID NO:145 or a fragment or a variation thereof;

amplifying a region of said template using said forward primer or said reverse primer to produce a product from said specimen; and

comparing said a product from said specimen with a nucleotide sequence from databases to determine said bacterium species present in the specimen.